



THE PLANKTON NEWS



THE NEWSLETTER OF THE SOUTHEAST PHYTOPLANKTON MONITORING NETWORK

Volume 4, Issue 2

June 2005

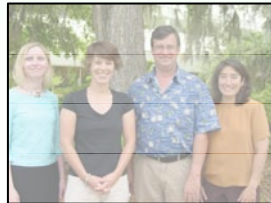
Extra! Extra!

First Flight High School Reports North Carolina's first algal bloom!

Note from SEPMN Staff:

We at SEPMN bring you this special edition of the Plankton News. We have exciting news to share! In April, a NC high school reported a *Pseudo-nitzschia* spp. bloom. We decided to use this issue to educate the public on what happens in the lab when a potentially toxic bloom is reported. In this issue, we will highlight some of the researchers and their jobs, as well as report the laboratory results of the tests that were conducted on this species. We send out our appreciation to all of our volunteers and supporters; the work you do is important and appreciated! Thank you!

Steve Morton, Project Lead
Wendy Wicke, Coordinator
Julie Cahill, Outreach Specialist
Kimberly Nowocin,
Web Site Coordinator



Science teachers, Leslie Horne and Katie Neller, and 12 students of First Flight High School in Kill Devil Hills, NC reported their very first phytoplankton bloom. This group began monitoring for the Southeast Phytoplankton Monitoring Network (SEPMN) in February 2005. On April 6, 2005, the group collected their sample from the FRF Pier in Duck, NC. Environmental conditions during that day for the site were as follows: salinity level at 25 ppt, water temperature at 13.1°C, air temperature at 70°F and winds were steady from the south. The group reported that the water color was a distinct green, not seen in previously collected samples.

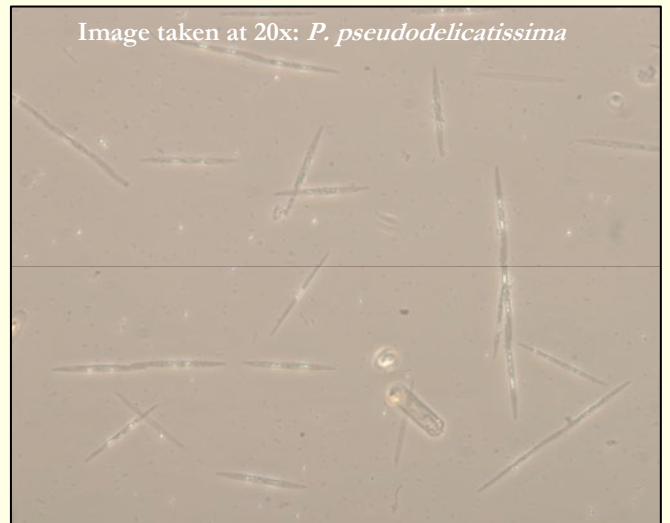
On Thursday, April 7, 2005, the students examined the sample under a microscope and immediately reported a possible bloom of *Odontella*, *Rhizosolenia* and some *Pseudo-nitzschia*. SEPMN staff instructed Leslie and Katie to send a preserved sample to the NOAA Marine Biotoxins Program in Charleston, SC. Laurinda Smith, a NOAA biologist, examined the sample under a scanning electron microscope (SEM) and found it to contain a bloom of *Pseudo-nitzschia pseudodelicatissima*. Utilizing the SEM is the only way to identify this particular species.

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Image taken at 20x: *P. pseudodelicatissima*



FFHS reports algal bloom, Continued from page 1

The identification of *P. pseudodelicatissima* is very exciting because it is the first time it has ever been reported in the Carolinas. Currently, *P. pseudodelicatissima* has only been reported in the Bay of Fundy, Gulf of Maine, Gulf of Mexico, and the Indian River Lagoon in Florida. This species has the potential to produce the toxin domoic acid, which can contribute to the human health hazard known as Amnesic Shellfish Poisoning (ASP).

After NOAA confirmed the species of phytoplankton, First Flight High School continued to report *Pseudo-nitzschia* blooms. The school sent us a 2-liter live sample so that we could conduct testing to see if the phytoplankton carried domoic acid. Unfortunately, the sample died enroute to our facility.

On April 25, 2005, Julie Cahill, SEPMN outreach specialist, traveled to NC to talk to our student volunteers about phytoplankton. While there, Julie collected additional live phytoplankton samples and shipped them to our laboratory. Three weeks after the initial report, NC still had a *Pseudo-nitzschia* spp. bloom. The sample Julie sent was tested for the toxin domoic acid. All testing results will be reported at the end of this newsletter.

As Julie traveled down the coast of NC, she stopped at various sites and collected additional samples. The *Pseudo-nitzschia* bloom stretched from northern Outer Banks, through Oregon Inlet near Manteo, and southward to Cape Hatteras. Leslie Horne, Katie Neller, and their students are continuing to monitor their site regularly. NOAA's Marine Biotoxins Program is conducting further studies of this diatom and will report any new information in future issues of the Plankton News. Congratulations to First Flight High School for this very important scientific discovery!



Photo credit: Susan Sullivan,
First Flight High School

Dedicated First Flight High School students sample in 28°F rainy weather at the FRF Pier in Duck, NC.

Species Spotlight

Pseudo-nitzschia spp.



Pseudo-nitzschia spp. is a genus of marine pennate diatoms that are found in estuarine to open ocean environments worldwide. There are 25 known species, 10 of which are potentially toxic to marine mammals, birds, and humans.

Pseudo-nitzschia spp. typically blooms in the spring and summer; however, they are not limited to these seasons. These diatoms range from 50 to 175 μm (micrometers) in length, depending on species; the shape is similar to that of a feather or flattened football. This species is classically characterized as forming stepped-chain patterns after asexual reproduction when the mother and daughter cells loosen and slide along each other until the polar ends are fixed.

The cell wall, or frustule, is made of silica (glass) and each species of *Pseudo-nitzschia* has unique frustule orientations and patterns. The frustule has pores, fibulae, striae, valves, girdles, and a raphe which help identify the species of *Pseudo-nitzschia*.

The species reported by First Flight High School is *Pseudo-nitzschia pseudodelicatissima*. This organism is generally very small, about 50-140 μm in length and 1.5-3.4 μm in width. *P. pseudodelicatissima* is one of the ten potentially toxic species in this Genus. Distribution is worldwide; however, this is the first time *P. pseudodelicatissima* has been observed in the Carolinas.

Amnesic Shellfish Poisoning & Domoic Acid

Shellfish poisoning is what the medical community defines as symptoms associated with eating shellfish that are toxic to humans. Amnesic shellfish poisoning (ASP) is the only known shellfish poison caused by a toxic diatom (genus *Pseudo-nitzschia*). When *Pseudo-nitzschia* spp. reproduces in large numbers, or blooms, high concentrations of the toxin (domoic acid) build up in organisms such as mussels, oysters, and clams that consume phytoplankton. All toxic phytoplankton produce natural harmful substances. Though these organisms have been around millions of years, only recently has technology allowed us to actually understand these microscopic, single-celled organisms.

There are a variety of symptoms that a person could develop if they consume shellfish that contains domoic acid. Symptoms may include vomiting, nausea, diarrhea and abdominal cramps, all occurring within 24 hours. In more severe cases, neurological symptoms may develop within 48 hours, which include headache, dizziness, disorientation, loss of short-term memory, motor weakness, seizures, coma, and possibly death. Short term memory loss is permanent, and is the major symptom that contributed to the name Amnesic Shellfish Poisoning. This toxin seems to directly and immediately affect the brain after it is consumed. Domoic acid acts as a neurotransmitter and causes certain nerves in the brain to be triggered over and over. This constant triggering leads to degeneration or decay of the brain tissue that is responsible for short term memory.

Some scientists think that domoic acid is a waste product for the diatom. Domoic acid has no direct effects on mussels and other shellfish feeding on the contaminated diatoms; however, the toxin in shellfish can potentially be transferred to an organism that consumed the shellfish. Any organism that consumes phytoplankton as a major part of its diet, or any organism that eats potentially toxic shellfish can be a carrier of domoic acid.

It is also important to note that domoic acid metabolizes at a very slow rate. Some shellfish areas have been closed to harvesting for a year or more after a toxic bloom has occurred. The reason shellfish are a common carrier of ASP is due to their incredible ability to filter large amounts of water on a daily basis. Some adult shellfish can filter as much as 30 liters of seawater in 24 hours. Depending on the shellfish species, water temperature will need to be taken into consideration because warmer waters can induce feeding. In places like Canada and Maine, some shellfish slow down growth and feeding rates during winter months. Also, different species of shellfish accumulate, metabolize, and eliminate toxins at different rates. Marine mammals like sea otters and sea lions are commonly suspect carriers of ASP because their diet consists of fish and shellfish. Does that mean sea otters can't remember what they eat for breakfast? There have been documented cases of sea lions exhibiting odd behaviors of wandering into streets and having seizures after being exposed to toxic blooms of *Pseudo-nitzschia*.

Remember, any toxin can not be removed by cooking or freezing shellfish. What do you do if you think you have ASP? See your doctor immediately! There is no known cure for ASP, but flushing any toxin present from your system ASAP would be the best thing to do.

The extent of illness a person with ASP suffers will depend on several variables such as the concentration of domoic acid consumed by the organism, the time of year, how long the bloom lasted, and the age of the person consuming the toxic shellfish.



U.S. locations where cases of ASP have been reported.

References:

<http://www.whoi.edu/redtide/illness/illness.html>

<http://www.doh.wa.gov/ehp/sf/Pubs/DomoicAcid.htm>

<http://www.foodsafety.wsu.edu>

<http://www.uaf.edu/seagrant/issues/PSP/PSP.pdf>

Identifying *Pseudo-nitzschia* spp.

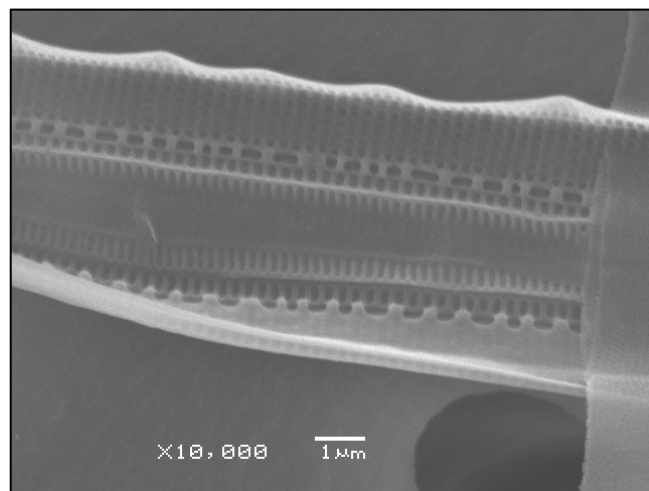
There are approximately 25 known species of *Pseudo-nitzschia*, 10 of which are harmful to the environment and humans. In order to differentiate between species, a scanning electron microscope (SEM) must be used.

A SEM is a microscope that uses electrons instead of light to form an image. To use the SEM, the sample must first be coated with a thin layer of gold so that it will conduct electricity and reflect electrons. The sample is then placed inside a vacuum column and air is pumped out. Inside the casing, there is an electron “gun” that emits a beam of high-energy electrons, a series of lenses to focus the electrons into a tight beam, and a scan coil that moves back and forth across the sample. As the electron beam moves over the sample, the electrons are reflected, picked up by the detector, amplified, and then fed to the monitor where the final image is displayed.

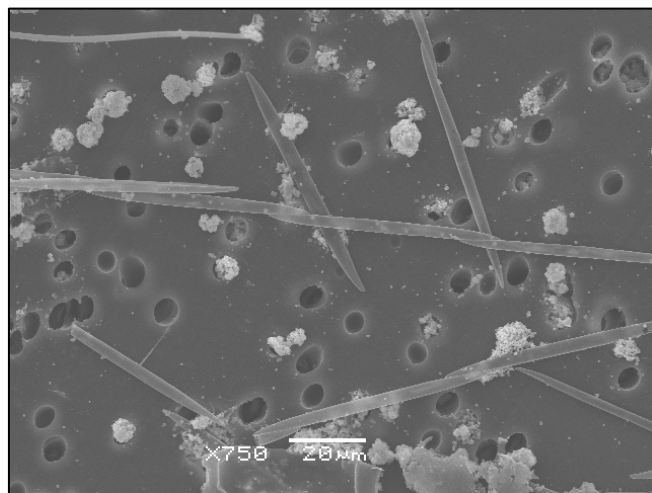
The SEM produces images at a very high resolution and level of magnification. A traditional light microscope produces images at a maximum of 1000x, whereas SEMs can magnify samples up to 300,000x. Most student microscopes can only magnify up to 40x. The purpose of an SEM is to visualize the surface morphology of an object or organism. The SEM also allows us to see the shape, size, and particle arrangement, as well as the elements and compounds that make up the sample.



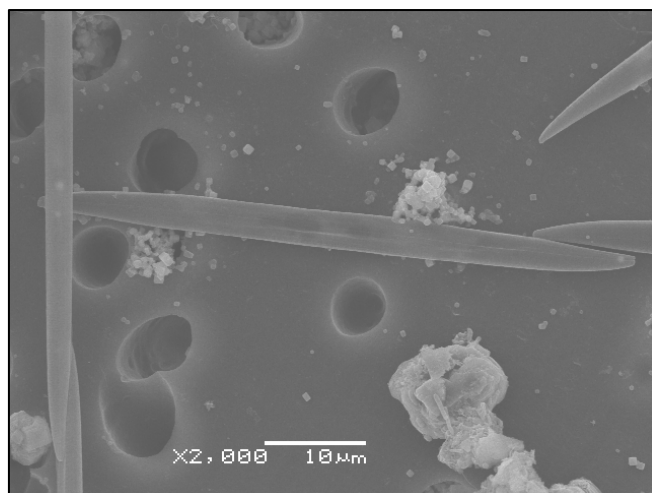
Laurinda Smith (above), a NOAA biologist, examined the sample from NC under a scanning electron microscope (SEM) and found it to contain a bloom of *Pseudo-nitzschia pseudodelicatissima*. The SEM allowed Ms. Smith to see the fine details such as the “ribs” or striae on the cell. The location, size, and orientation of the pores that are between the striae help determine what species of *Pseudo-nitzschia* was in the sample. Check out the SEM images of *P. pseudodelicatissima* in the next column.



Frustule of *Pseudo-nitzschia pseudodelicatissima*



Step-chain of *Pseudo-nitzschia pseudodelicatissima*



Single cell of *Pseudo-nitzschia pseudodelicatissima*

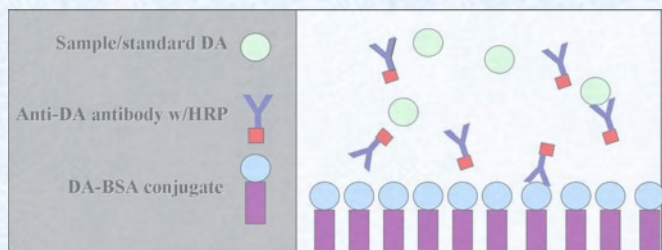
Domoic Acid ELISA

The dinoflagellate *Pseudo-nitzschia pseudodelicatissima* has the potential to produce the toxin domoic acid. However, in this species, domoic acid is very difficult to detect because it is usually in very low concentrations. Jennifer Maucher, a marine biologist with NOAA's Marine Biotoxins Program, conducted the domoic acid (DA) testing on the sample sent in from First Flight High School (FFHS).



The domoic acid test is called an ELISA or “Enzyme-Linked Immunosorbant Assay.” This test kit was developed by a company called BioSense in Norway.

The test kit consists of a 96-well plate that contains a DA-BSA conjugate (two different molecules stuck together) at the bottom of each well. BSA is a sticky protein that helps the domoic acid molecules stick to the bottom of the plate. To begin the experiment, Jennifer Maucher will add the *P. pseudodelicatissima* sample from NC and an anti-DA antibody labeled with HRP to the plate.



The HRP is an enzyme attached to the antibody that acts like a flag showing where the antibody has attached. The anti-DA antibody, with the HRP “flag”, will attach to either DA on the plate or to DA in the sample. After an hour of reaction time, the plate undergoes a “wash” of PBST buffer, which will remove all antibodies (and their flags) that are not attached to the DA on the plate. Finally, an enzyme called TMB is added and reacts with the flags of antibodies attached to the plate, which causes a color change in the well.

DA ELISA, continued.

If there is no DA in the sample, then the majority of flags bind to your plate, and the TMB causes a significant color change. Your sample is very dark in color. The test result is then negative for DA.

If there is a lot of DA in the sample, then the flags do not bind to your plate, but rather to DA in the sample. The buffer washes all the flags away, and the TMB added causes very little color change. Your sample is very light in color, and your test results are positive for domoic acid.

Once this test is completed, it is then compared to a standard. A standard is the same test done with known amounts of domoic acid. Each known amount of DA creates a specific color. The sample results are compared to the standard to get a matching color which tells us the concentration of DA within the sample. Check out the results section at the end of this newsletter!

Volunteer Spotlight

Blooming Scientists, Chelsea Elwang, Shawn Ruzzi, Jessica Kimsey, Aubrey Briggman, Logan Wilkerso, Caren Letchford, Katie Godwin, Kayla Gibbs, LeeAnn Swanner, Ryan Krall, Sara Ritenour, Kealey Newton, Kate Carbocci along with teachers Leslie Horne and Katie Neller are from First Flight High School in Kill Devil Hill, NC, learned how important their discovery was to NOAA scientists. When asked what they liked most about being a part of SEPMN, Ryan Krall said, “The most enjoyable part about the plankton research project is going on to the research pier in treacherous weather conditions”. Aaron Hoare is now interested in becoming a mycologist or marine scientist. “The plankton project has really motivated him; even his other teachers notice a difference in their classes since he started the program! Those are the rewards I live for!!” says Neller.



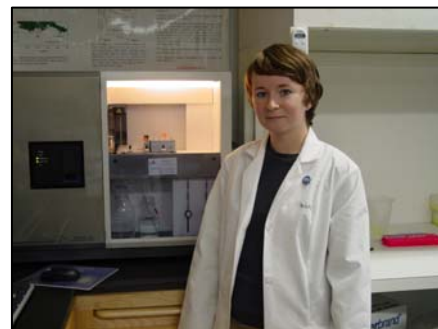
First Flight High School students and their teacher awarded with NOAA certificates of appreciation for their report of the *Pseudo-nitzschia* spp. bloom.

Domoic Acid Extractions

The water sample containing the *Pseudo-nitzschia pseudodelicatissima* can not be used immediately in any tests; it first must be prepared properly by separating the potentially domoic acid – containing diatoms from the rest of the sediment and water. Kristen King, a biologist with the Marine Biotoxins Program, conducted the domoic acid extractions that were used in the DA ELISA and DA SPR tests.

To begin, the particulate matter in the water sample is filtered onto glass fiber filters. The glass fiber filters are then grinded with a methanol solution. A sonicator (a machine that emits sound waves) is used to break up the siliceous cell walls of the diatoms to release any potential domoic acid.

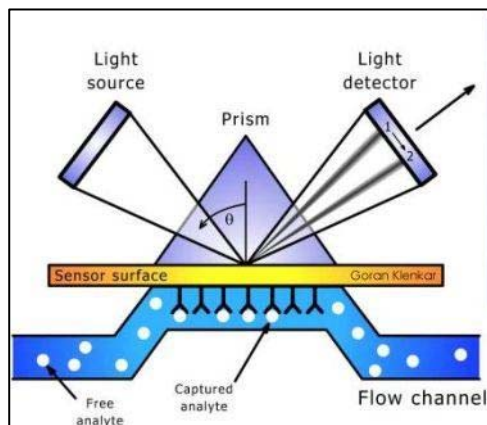
Once the cells walls of the diatoms are broken up, the sample is then spun down. The researcher then removes the supernatant (methanol and potential toxin) and filters it one final time. The sample is then ready to be used in the domoic acid ELISA and SPR tests.



Surface Plasmon Resonance (SPR)

SPR or surface plasmon resonance is a complicated testing method similar to ELISA. SPR is new technology pioneered by a company called BIACORE based in Sweden. The technology was initially developed for food analysis to check for proteins, antibiotics, hormones, and chemicals in milk. Colleagues in Northern Ireland use the SPR to test shellfish for regulatory purposes and the Marine Biotoxins Program tailored their SPR methods and protocol for research in harmful algal blooms. The test is completely automated but was monitored by Kristen King.

According to BIACORE, SPR is an electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions. Let's simplify that a bit.



Domoic acid is immobilized on the underside of a sensor chip (about the size of a microscope slide). There is a flow channel underneath the sensor chip in which the domoic acid extraction (water sample from First Flight High School) will travel through. The domoic acid on the sensor chip competes with the domoic acid in the sample for available antibodies mixed with the sample. Any antibody that does not bind to the DA in the sample will bind to the DA on the sensor chip instead. A beam of light is shined onto the sensor chip and the angle of refraction (the angle in which the light reflects back) is measured. Any binding event to the sensor chip increases the angle of refraction. This data (the angle of refraction) is converted into concentration of toxin based on a calibration curve also produced by the instrument. The greater the angle of refraction of light means less concentration of domoic acid in the sample.

Ask the Scientist

What triggers the diatom to start producing the toxin?

Aaron Hoare 10th grade First Flight High School

Answer: This is a really great question! Some blooms of *Pseudonitzschia* are known to produce domoic acid; a neurotoxin to humans and marine mammals. However, the triggers that induce toxin production by toxin-producing harmful algae such as *Pseudonitzschia* sp. are largely unknown but of great interest to marine biologists and others. To make matters even more complicated, toxin production can not only be triggered 'on', but can also be triggered to turn 'off'. Years of research using multiple species of harmful algae have explored the possibilities of a combination of both genetic and environmental triggers that control toxin production. Probably the hypothesis that has gathered the greatest attention is the link between toxin production and nutrient stress. Algae need nutrients for growth. However, when nutrients such as nitrate, phosphate, iron and sometimes silicate become low in the water, there is often an increase in toxin production by the algae. It is thought that the toxins may give the algae an ecological advantage during these periods of low nutrient stress. It may be proven to be a true case of survival of the fittest!

Michael Twiner, Ph.D. Marine Biotoxins Program, NOAA/NOS

Do some shellfish take longer to rid themselves of the toxin? Barry King, Aquaculture major Carteret Community College

Answer: YES, The topic of detoxification is important to the harmful algal bloom (HAB) field in many ways, but probably the most costly pertains to the fisheries and shellfish industries. It is quite simple, the longer a shellfish stays toxic the longer it can infect other species higher on the food chain, and also the longer it will be before "Joe Fisherman" can harvest those shellfish to make the almighty dollar.

The rate of toxin depuration depends on not only the shellfish, but also the toxin they are exposed to. The rate of toxin elimination is highly dependant on the toxin's mode of action, solubility, and the site of toxin storage. Not only that true, but toxin elimination can depend on the size of the shellfish, the temperature of the water, the tissue makeup of the shellfish, and heck some shellfish just refuse to eat toxic algae!

Ricky Woofter, Marine Biotoxins Program, NOAA/NOS

Why doesn't the toxin kill the shellfish?

Allen Sutton 7th grade, Broad Creek Middle School

Answer: Great question, some shellfish actually do die from the toxins (spiny scallops), so it kind of depends on the species of shellfish. There isn't very much information about exactly why shellfish don't die, but we think that it has to do with the receptor that the DA binds to being a different shape in the shellfish so the DA doesn't bind to it, but does in humans. While in some cases these toxins do not kill the shellfish, they can affect the physiology, for example; alter feeding rates and reproduction.

Karen Mao, Lab Technician. Marine Biotoxins Program, NOAA/NOS

If you have a question about toxic phytoplankton we will post it in the next edition. Send your question, name, grade and school to The Plankton News. See page 9 for mailing address.

SEPMN would like to give special thanks to our young scientists at First Flight High School and the scientists from the Marine Biotoxin Program for helping us bring you this special edition of the Plankton News!

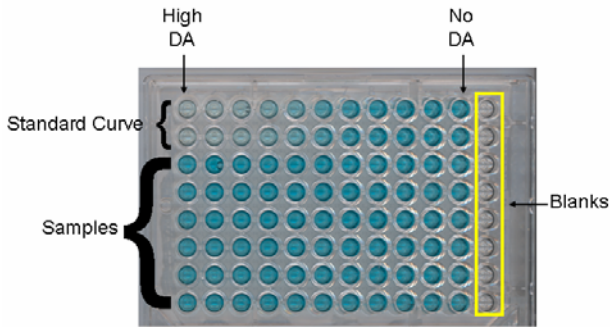
Potentially toxic genera

SEPMN is especially interested in getting preserved and live samples of these potentially toxic genera. If you have a significant number of these species in your sample (common, abundant, or bloom), contact us immediately.



Results

The ELISA told us that DA was not present in the *Pseudo-nitzschia* bloom. The plate below shows us that all the wells containing the sample (bottom 6 rows) were blue. When these are compared to the standard curve wells (shown in the first 2 rows), they matched those that signify no DA, thus there was no DA present in any of the samples tested.



The SPR data also revealed a lack of DA in the phytoplankton sample from NC. The instrument measured a very large angle of refraction for each of the samples, which is indicative of undetectable levels of DA. We are going to continue to test for the production of Domoic Acid.

Since live material was shipped to the Marine Biotoxins Program, cultures of *P. pseudodelicatissima* were initiated. From this collection, Steve Morton was able to isolate 5 cultures. These cultures will be grown to a 1 Liter volume and tested for the possible production of DA just like the bloom samples. These cultures are also going to help an ongoing biogeographical study by international researchers on the genetic variability of the genus *Pseudo-nitzschia*.



Glossary of Terms

Asexual reproduction- Dividing of one cell onto two to four cells.

Diatoms- Any of various microscopic one-celled or colonial algae of the class Bacillariophyceae, having cell walls of silica consisting of two interlocking symmetrical valves.

Fibulae- In some pinnate diatoms, the internal silica bridges that subtend the raphe.

Frustule- In diatoms, the siliceous parts of the cell wall.

Girdle- Part of the frustule between the valves and connecting bands.

Micrometer (μm)- A metric unit of length equal to one millionth (10⁻⁶) of a meter.

Morphology- The form and structure of an organism or one of its parts without consideration of function.

Pennate- Diatoms with the valve striae arranged in relation to a line.

Pores- Small holes that can be for excreting mucus.

Raphe- One or two shaped slits arranged end to end along or around the valve margins.

Stepped-chained pattern- A series single celled organisms overlapping in a stair like formation.

Striae- Striation or a line of pores.

Valves- The intricately sculptured units of the cell wall lying at each end of the cell.

References:

Horner, Rita A. 2002. A Taxonomic Guide To Some Common Phytoplankton. Biopress Limited, Dorset Press, Dorchester, UK.

<http://dictionary.reference.com/>

Calendar of Events

National Marine Educators' Association "Look to the Source, Look to the Sea"

Annual Conference

Kahului, Maui, Hawai'i

July 11-16, 2005

Fun with Phytoplankton, a 3-hour hands-on workshop

Georgia Annual Refresher Training Course

Burton 4-H Center, Tybee Island, GA

Potluck Dinner, July 29, 2005 at 6 pm (Lodging available)

Training Course, July 30, 2005 from 8:30 am – 3 pm

(Breakfast and lunch provided)

South Carolina Annual Refresher Training Course

NOAA's Center for Coastal Environmental Health &

Biomolecular Research Building

Fort Johnson Marine Complex, Charleston, SC

August 6, 2005 from 8:30 am – 3 pm

(Breakfast and lunch provided)

North Carolina Annual Refresher Training Courses

US Army Core of Engineers Research Facility

Duck, NC

August 13, 2005 from 8:30 am – 3 pm

(Breakfast and lunch provided)

Carteret Community College

Morehead City, NC

August 14, 2005 from 8:30 am – 3 pm

(Breakfast and lunch provided)

Ft. Fisher Aquarium

Kure Beach, NC

August 26, 2005 from 8:30 am – 3 pm

(Breakfast and lunch provided)

THE PLANKTON NEWS

Direct all correspondence to:

Southeast Phytoplankton

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NOAA /NOS/

Marine Biotoxins Program

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Reporting a Bloom

If you and your group think you might have a phytoplankton bloom, please contact SEPMN staff **immediately** via phone or email. Make as many observations as you can about your site: weather conditions, water coloration, tides, and currents. Record them on your data sheet. If you have a camera, we would love pictures of your site during a bloom.

Depending on the species of phytoplankton, you may need to send us a preserved and/or a live sample. The students and teachers from First Flight High School needed to send us both. Due to First Flight High School's rapid response, we were able to identify the species within 24 hours of the bloom. We also began culturing the diatom in our labs for further studies.

Preserved Sample

After you determine that you have a bloom, pour some of the sample into a separate clean bottle and add Lugol's solution. Do not use the sampling bottles; you want to keep them clean from stains or chemicals for future samplings. Only a small amount of Lugol's solution is needed to preserve your sample (1% - 2% dilution). For example, add 1 mL of Lugol's solution to a 50 mL sample. Place your preserved sample into a zip lock bag and then pack it into a padded envelope or small box. The sample should be shipped via Federal Express Priority Overnight to our office. Please contact us prior to shipping; we will pay for the cost of shipping.

******Please keep all of your receipts. SEPMN will reimburse you for all of your purchases related to shipping materials and fees.***

Live Sample

We will let you know if we need a live sample from your site. *Live samples need to be shipped immediately after collection.* When collecting a live sample, there is no need for a plankton tow. Just use a clean container with a cap or lid and take a direct water sample. We may ask for a 2-liter sample; cleaned soda bottles make great containers. Label your sample with the group name, location of sampling site, and the date and time of the sample taken. You will need to ship the sample in a small cooler with packing ice (blue ice or frozen water bottles). Place the packing ice on the bottom of the cooler. Put a space or buffer (bubble wrap, packing peanuts, or newspaper) between the ice and the sample to keep it cool and prevent it from freezing. Again, ship the sample to us via Federal Express Priority Overnight.



Southeast Phytoplankton Monitoring Network (SEPMN)
NOAA/NOS/Marine Biotoxins Program
Center for Coastal Environmental Health and Biomolecular Research
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